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In vivo Safety of Dichloromethane-Methanolic Extract of *Allium sativum* in Normal Mice

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Abstract

The indiscriminate use of *Allium sativum* in treatment of various diseases can pose a great danger to various body functions. Assessment of the effect of the extract on haematological and biochemical parameters can be used to explain the blood, liver and kidney related functions. Sub-chronic toxicity evaluation was done by orally administering extracts dissolved in 1% DMSO, using a drenching syringe in doses of 100, 500, 1000 and 200 mg/kg body weights of extract for 28 days. The control group was given DMSO at the same frequency and period. The body weights of both treated and control rats was recorded before, during and at the end of the experiment. The results of this study showed that dichloromethane-Methanolic extract of *A. sativum* induced significant increase in the levels of red blood cell, haemoglobin and haematocrit across the 100, 500, 1000 and 2000 mg/kg.bw dose levels ($p<0.05$). However, the red blood cell indices did not show a significant change at all the dose levels ($p>0.05$). The total and differential white blood cell counts also increased significantly at all dose levels ($p<0.05$). However, the platelets and the related parameters did not have any significant change at all the dose levels during this study period ($p>0.05$). The dichloromethane-Methanolic extract of *A. sativum* caused a significant increase in the levels of liver functions profiles across the 100, 500 and 2000 mg/kg.bw dose levels ($p<0.05$). Qualitative phytochemical screening confirmed the presence of various phytochemicals which included alkaloids, flavonoids, steroids, saponins, cardiac glycosides and phenolics. These phytochemicals are understood to play a major role in gene expression, erythropoietin stimulatory, thrombopoietic stimulatory, immune-stimulatory and enzyme activities. Flavonoids, cardiac glycosides and alkaloids are also responsible for the increase in the red blood cell count through their antioxidant properties. Conversely, an increase in saponins may result to a decrease in red blood cell indices. It was therefore concluded that the plant extract, subject to various stipulated assays, is safe at particular doses as indicated by changes in haematological parameters. On the other hand, the study also shows that the plant extract is not safe at high doses as indicated by change in the liver and kidney parameters which showed hepatotoxicity and nephrotoxicity respectively.

Keywords: *Allium sativum*; Extracts; Phytochemicals; *In vivo* safety

Introduction

Garlic also known as *Allium sativum* has been one of the oldest cultivated plants worldwide. It has been used in a variety of ways as food, spice and conventional medicine for over 4000 years [1]. The garlic plant has been included in about 22 therapeutic formulations which make it an effective remedy for a variety of ailments including heart problems, headache, bites, tumours and also worms [2].

According to the US Food and Drug Administration survey of 900 people, it was found out that approximately 17% of the population utilized a garlic supplement in the preceding 12 months [3]. This plant has been appreciated for its unique functions such as anti-microbial, hypolipidemic [4], anticarcinogenic [5], anti-viral, anti-bacterial, antifungal [6], anti-atherosclerotic and antioxidant [7] capacities. There are also additional functions that garlic has been reported to have including anti-atherosclerotic and anti-cancer properties.

Garlic was involved in the pasteurization of milk due to its antibacterial properties as was reported by the famous chemist and microbiologist Louis Pasteur [2]. In the past centuries in India, garlic was used as antiseptic lotion for wound washing and ulcers. Moreover, soldiers of war during the World War II were treated with garlic in order to increase wound healing [8].

A. sativum as an alternative medicine for a wide range of ailments such as bacterial, cancer, viral and viral infections is economically viable as compared to the conventional drugs. However, the indiscriminate use of *A. sativum* in the treatment and management these diseases pose a great risk to patients. It is important to understand and scientifically test how much in terms of concentration of the herb is safe and what is unsafe. There has been no scientific data and safety margin on the *in vivo* safety of DCM-MeOH extract of *Allium sativum*. Disturbances and changes in body weight of the animals, haematological and biochemical parameters were indicators of either safety or toxicity in the assay.

Materials and methods

Collection and preparation of plant material

Garlic (*Allium sativum*) was collected from Kiambu market in Kiambu county, central province. A voucher specimen was deposited in the Kenyatta University herbarium. The plant material was washed thoroughly in tap water and put in liquid nitrogen, and then grounded

into small particles using a pestle and mortar. The powdered samples were weighed, labeled and recorded [9,10].

Extraction

A 200 g measure of the powdered extract of the plant material was put in a beaker and DCM-MeOH mixture in 1:1 ratio added in order for the dry powder to be submerged in the solvent. The mixture was allowed to soak for 24 hr. The mixtures were then decanted, filtered using muslin cloth and then with Whatman filter paper No. 1. The whole process was repeated three times and the supernatant collected and pooled together. The extract was concentrated by rotary evaporator at 40°C to obtain the dry extract as described by Evans [9].

Laboratory animals and experimental design

Thirty healthy mice aged between 8 and 12 weeks were obtained from the Department of Biochemistry and Biotechnology animal house. They were then kept under standard environmental conditions 25°C, 12 hr light and 12 hr dark cycle and were supplied with standard pellet diet and water ad libitum. The mice were allowed to acclimatize for a period of 7 days.

The control group received DMSO while the experimental groups received extracts dissolved in 1% DMSO orally, using a drenching syringe, in doses of 100, 500, 1000 and 2000 mg/kg body weights of the extract for 28 days. The mice were observed daily for any abnormal clinical signs and in case of mortality the mean lethal dose (LD50) was to be established. Their body weights were taken at the end of every week.

Following overnight fasting on the 29th day, all animals were sacrificed and 6 ml blood sample was collected through cardiac puncture using a sterile syringe from each mouse. Three millilitre portion of the blood was put in bottles containing EDTA to prevent coagulation. The EDTA blood samples were immediately used to determine haematological parameters (full haemogram) using automated analyzer (Model: Changchun Dirui Industrial Co., LTD). The remaining 3 ml blood sample for biochemical tests was collected in bottles without EDTA and kept at 4°C for 4 hr to let it clot and centrifuged at 1500 rpm for 15 min to obtain serum. The serum was refrigerated at -22°C and used for biochemical assays.

Determination of haematological parameters

A full haemogram was conducted using a chemistry auto analyzer. It was analysed following the protocol described by Hayes [11]. Erythrocytic parameters that were analysed included the hemoglobin count (HB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), and mean cell volume (MCV) and red cell distribution with (RDW). The leukocytic parameters compromised of the percentage neutrophils (%NE), percentage lymphocyte (%LYM), percentage eosinophils (%EO), percentage basophils (%BA) and percentage monocyte (%MON). While platelets parameters were the platelets count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW).

Determination of biochemical parameters

Liver and kidney function tests were investigated by an automated chemistry analyzer as outlined by Hayes [11]. The liver function markers of importance included alanine aminotransferase (ALT), total bilirubin (TBILI), aspartate aminotransferase (AST) and direct bilirubin (DBILI). Kidney function markers included Blood urea nitrogen (BUN), creatinine and electrolytes (Cl^-, K^+ and Na^+). The electrolytes were analysed using an electrolyte panel.

Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by Harbone [12]. The secondary metabolites tested included flavonoids, terpenoids, cardiac glycosides, alkaloids, steroids, tannins, saponins and phenols.

Data management and statistical analysis

Experimental data on effect of treatment with *A. sativum* extract over time was compared between the control group and the treated groups, recorded and tabulated on a broad sheet using Ms Excel program. Analysis of the data was done using Minitab statistical software. The results were expressed as mean ± standard error of mean (SEM) for analysis. Statistical significance of difference among groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise separation and comparison of means. $P \leq 0.05$ was considered significant.

Results

Effect of DCM-MeOH extract of *Allium sativum* on body weight in normal mice

It was observed that an increase in the concentration of DCM-MeOH extract of *A. sativum* led to a significant change in body weight of the experimental group in comparison to the control group ($p<0.05$; Table 1).

In the first week of the extract administration, there was an insignificant change in body weight of mice in control group compared with mice in all the experimental groups (100, 500, 1000 and 2000 mg/kg.bw) ($p>0.05$; Table 1). In the second week of extract administration at the four doses, there was significant increase in body weight of mice in the control group compared with the mice in groups administered with the extract at doses of 500, 1000 and 2000 mg/kg.bw in a dose-dependent manner ($p<0.05$; Table 1). However, the change in body weight of mice in the group administered with 100 mg/kg.bw dose of the extract was insignificant compared with the control group ($p>0.05$; Table 1). In addition, there was no significant difference in the body weight of mice administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 1). Similarly, there was an insignificant change in body weight of mice administered with the extract at doses of 1000 and 2000 mg/kg.bw ($p>0.05$; Table 1)

In the third and fourth week of extract administration at various doses, there was a significant change in body weight of mice in the control group compared with mice in the four experimental groups ($p<0.05$; Table 1). However, the weight levels were not reduced in a dose-dependent manner. Moreover, the change in body weight among the mice in the experimental group was not significantly different ($p>0.05$; Table 1).

Treatment	Weekly weight of mice (g)					Δ Weight/Week
(mg/kg.bw)	0	1	2	3	4	(g/Week)
Control	19.40 ± 0.51^{a}	20.08 ± 0.32^{a}	20.54 ± 0.41^{a}	21.64 ± 0.36^{a}	22.62 ± 0.41^{a}	0.81 ± 0.19^{a}
100	19.40 ± 0.60^{a}	18.10 ± 0.95^{a}	19.54 ± 0.90^{ab}	18.00 ± 0.69^{b}	18.34 ± 1.43^{b}	-0.27 ± 0.27^{b}
500	18.60 ± 0.40^{a}	17.64 ± 0.86^{a}	17.94 ± 0.41^{b}	17.14 ± 0.36^{b}	17.32 ± 0.73^{b}	-0.32 ± 0.20^{b}
1000	19.40 ± 0.51^{a}	18.20 ± 0.81^{a}	17.90 ± 0.63^{c}	17.16 ± 0.29^{b}	17.56 ± 0.55^{b}	-0.46 ± 0.14^{b}
2000	19.20 ± 0.25^{a}	17.86 ± 0.22^{a}	17.08 ± 0.20^{bc}	17.38 ± 0.15^{b}	17.26 ± 0.31^{b}	-0.49 ± 0.13^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤ 0.05 (Analysed by ANOVA followed by Tukey's post hoc test). Key: Δ: represents change in

Table 1: Effect of oral administration of DCM-MeOH extract of *Allium sativum* on body weight of normal mice.

There was significant increase in the average weight change per week of mice in the control group compared with mice in the experimental groups ($p<0.05$; Table 1). However, the average weight change per week among mice in the experimental groups was not significantly different ($p>0.05$; Table 1).

Effect of DCM-MeOH extract of *Allium sativum* on erythrocytic parameters in normal mice

There was significant increase in RBC count in mice administered with the extract at a dose of 2000 mg/kg.bw compared with mice in the control group ($p<0.05$; Table 2).

However, there was insignificant change in RBC count in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 2). Moreover, there was no significant change in RBC count in mice administered with the extract at doses of 500, 1000 and 2000 mg/kg.bw ($p>0.05$; Table 2). The increase in RBC count was dose-dependent among the experimental groups.

Similarly, there was a significant increase in HB levels in mice in the control group compared with mice administered with the extract at a dose of 100 mg/kg.bw, whereas, there was a significant decrease in HB levels in mice in the control group compared to mice administered with the extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 2). In addition, there was insignificant change in HB levels in mice in the control group compared with the mice administered with the extract at doses of 500 and 1000 mg/kg.bw ($p>0.05$; Table 2). However, the HB levels were not increased in a dose-dependent manner.

Treatment	Erythrocytic Parameters						
(mg/kg.bw)	RBC	HB	HCT	MCV	MCH	MCHC	RDW
Control	6.31 ± 0.19^{b}	9.64 ± 0.49^{b}	34.40 ± 1.38^{a}	54.52 ± 0.75^{a}	14.22 ± 0.25^{a}	26.54 ± 0.36^{a}	17.08 ± 0.50^{a}
100	7.30 ± 0.65^{b}	8.72 ± 0.27^{c}	36.28 ± 1.31^{ab}	52.34 ± 4.69^{a}	13.80 ± 2.06^{a}	25.58 ± 1.29^{a}	17.68 ± 0.56^{a}
500	7.64 ± 0.53^{ab}	9.88 ± 0.76^{ab}	38.90 ± 2.92^{ab}	53.12 ± 1.18^{a}	12.96 ± 0.43^{a}	24.74 ± 0.23^{a}	17.66 ± 0.99^{a}
1000	7.78 ± 0.74^{ab}	9.42 ± 1.08^{ab}	39.10 ± 6.03^{b}	53.26 ± 0.49^{a}	13.42 ± 0.56^{a}	24.42 ± 0.58^{a}	16.56 ± 0.56^{a}
2000	7.88 ± 0.41^{a}	10.10 ± 0.65^{a}	40.80 ± 2.35^{b}	53.86 ± 0.82^{a}	13.88 ± 0.27^{a}	25.34 ± 0.48^{a}	15.90 ± 0.38^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at p ≤ 0.05. (Analysed by ANOVA followed by Tukey's post hoc test). Key: RBC: red blood cell count, HB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width

Table 2: Effect of DCM-MeOH extract of *A. sativum* on Erythrocytic parameters in normal mice.

There was a significant decrease in HCT count in mice in the control group compared with the mice administered with the extract at doses of 1000 and 2000 mg/kg.bw ($p<0.05$; Table 2). There was also an insignificant change in HCT count in mice in the control group compared with mice administered with the extract at doses of 100 and 500 mg/kg.bw ($p>0.05$; Table 2). Moreover, there was no significant change in HCT count in mice administered with the extract at doses of 100, 500, 1000 and 2000 mg/kg.bw of the extract ($p>0.05$; Table 2).Increase in the HCT count was in a dose-dependent manner.

However, the DCM-MeOH extract of *A. sativum* did not have any significant change in MCV, MCH, MCHC and RDW profiles in mice in the control group compared with the mice in all the experimental groups after the 28-days ($p>0.05$; Table 2). In addition, the MCH and MCHC levels were not reduced in a dose-dependent manner. However, the MCV and RDW levels were reduced in a dose-dependent manner.

Effect of DCM-MeOH extract of *Allium sativum* on leukocytic parameters in normal mice

There was a significant decrease in WBC count in mice in the control group compared with mice administered with extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 3). However, there was no significant change in WBC count in mice in the control group compared with mice administered with the extract at doses of100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 3). Similarly, there was an insignificant change in WBC count among mice administered with the extract at doses of 1000 and 2000 mg/kg.bw ($p>0.05$; Table 3). The increase in WBC count was dose-dependent.

Treatment	Leukocytic Parameters					
(mg/kg.bw)	WBC	NE	LY	MO	EO	BA
Control	8.26 ± 1.30^{a}	1.08 ± 0.42^{a}	3.35 ± 0.17^{b}	0.28 ± 0.12^{a}	0.00 ± 00^{a}	1.50 ± 0.19^{a}
100	8.56 ± 2.28^{a}	1.36 ± 0.23^{ab}	4.84 ± 0.48^{a}	0.66 ± 0.48^{a}	0.02 ± 0.02^{a}	0.76 ± 0.14^{b}
500	8.02 ± 0.35^{a}	1.38 ± 0.86^{ab}	4.94 ± 0.45^{a}	0.04 ± 0.25^{a}	0.00 ± 0.00^{a}	0.82 ± 0.17^{ab}
1000	9.05 ± 1.69^{ab}	1.46 ± 0.28^{ab}	7.26 ± 1.70^{a}	1.12 ± 0.44^{a}	0.00 ± 0.00^{a}	1.14 ± 0.18^{ab}
2000	11.26 ± 0.94^{b}	1.54 ± 0.22^{b}	7.70 ± 0.61^{a}	0.44 ± 0.25^{a}	0.00 ± 0.00^{a}	0.74 ± 0.18^{b}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$. (Analysed by ANOVA followed by Tukey's post hoc test). Key: WBC: white blood cell count, NE: neutrophil count, LY: lymphocyte count, MO: monocytes, EO: eosinophils, BA: basophils

Table 3: Effect DCM-MeOH extract of *Allium sativum* on Leukocytic counts in normal mice.

There was a significant decrease in neutrophil count in mice in the control group compared with mice administered with the extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 3). It was also observed that there was no significant change in neutrophil count in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 3). There was also insignificant change in neutrophil count among mice in all the experimental groups ($P>0.05$; Table 3). There was a dose-dependent manner in the increase of neutrophil count.

There was a significant increase in lymphocyte count in mice administered with the extract at doses of 100, 500, 1000 and 2000 mg/kg.bw compared with the mice in the control group ($p<0.05$; Table 3). However, there was no significant change in lymphocyte count among mice in all the experimental groups ($p>0.05$; Table 3). Lymphocyte count increase was dose-dependent.

The DCM-MeOH extract of *A. sativum* did not show a significant change in monocyte and eosinophil count in mice in the control group compared with mice in the experimental groups (100, 500, 1000 and 2000 mg/kg.bw) ($p>0.05$; Table 3). However, the monocyte and eosinophil count were not increased in a dose-dependent manner.

Conversely, there was a significant increase in basophil count in mice in the control group compared with mice administered with the extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 3). There was also a significant increase in basophil count in mice in the control group compared with the mice administered with the extract at a dose of 100 mg/kg.bw ($p<0.05$; Table 3). However, there was an insignificant change in basophil count in mice in the experimental groups ($p>0.05$; Table 3). The basophil count did not reduce in a dose-dependent manner.

Effect of DCM-MeOH extract of *Allium sativum* on Platelets and their related parameters in normal mice

Treatment	Thrombocytic Parameters			
(mg/kg.bw)	PLT	PCT	MPV	PDW
Control	947.00 ± 131.00^{a}	0.29 ± 0.05^{a}	3.42 ± 0.28^{a}	14.88 ± 0.69^{a}
100	865.00.4 ± 66.30^{a}	0.38 ± 0.05^{a}	4.08 ± 0.63^{a}	14.32 ± 0.37^{a}
500	1014.0 ± 108.00^{a}	0.37 ± 0.04^{a}	3.20 ± 0.24^{a}	14.32 ± 0.37^{a}
1000	1131.00 ± 166.00^{a}	0.32 ± 0.05^{a}	3.66 ± 0.35^{a}	14.56 ± 0.49^{a}
2000	986.60 ± 80.50^{a}	0.38 ± 0.05^{a}	3.46 ± 0.28^{a}	14.90 ± 0.70^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$ (Analysed by ANOVA followed by Tukey's post hoc test). Key: PLT: platelet count, PCT: platelet crit, MPV: mean platelet volume, PDW: platelet distribution width

Table 4: Effect of DCM-MeOH extract of *A. sativum* on Platelets and their related parameters in normal mice.

There was no significant change in PLT, PCT, MPV and PDW levels in mice in the control group compared with mice in all the experimental groups ($p>0.05$; Table 4). In addition, the PLT, PCT and MPV levels were not increased in a dose-dependent manner. However, the PDW levels were increased in a dose-dependent manner.

Effect of DCM-MeOH extract of *Allium sativum* on liver functions in normal mice

There was a significant increase in ALT levels in mice in the control group compared with mice administered with the extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 5). However, there was no significant change in ALT levels in mice in the control group compared with mice administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($P>0.05$; Table 5). The ALT levels were not increased in a dose-independent manner.

Moreover, there was a significant increase in AST levels in mice in the control group compared with mice administered with the extract at doses of 100, 1000 and 2000 mg/kg.bw ($p<0.05$; Table 5). There was also a significant increase in AST levels in mice administered with the extract at a dose of 2000 mg/kg.bw as compared with mice administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($p<0.05$; Table 5). In addition, there was no significant change in AST levels in mice in the control group compared with mice administered with the extract at a dose of 500 mg/kg.bw ($p>0.05$; Table 5). Similarly, there was an insignificant change in AST levels in mice administered with the extract at doses of 500 and 1000 mg/kg.bw ($p>0.05$; Table 5). Moreover, the AST levels were not increased in a dose-dependent manner.

Treatment	Liver functions Parameters			
(mg/kg.bw)	ALT	AST	TBILI	DBILI
Control	35.48 ± 1.31^{a}	67.00 ± 3.79^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
100	52.50 ± 3.82^{ab}	213.00 ± 22.10^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
500	38.80 ± 4.66^{a}	162.20 ± 16.50^{ab}	0.12 ± 0.03^{b}	0.05 ± 0.05^{ab}
1000	55.00 ± 18.80^{ab}	247.10 ± 59.90^{b}	0.13 ± 0.02^{ab}	0.08 ± 0.03^{ab}
2000	62.00 ± 19.50^{c}	288.00 ± 65.00^{c}	0.52 ± 0.16^{a}	0.08 ± 0.02^{a}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$. (Analysed by ANOVA followed by Tukey's post hoc test). Key: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBILI: Total bilirubin; DBILI: Direct bilirubin.

Table 5: Effect of DCM- MeOH extract of *A. sativum* on liver functions in normal mice.

There was a significant decrease in TBILI levels in mice in the control group compared with the mice administered with the extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 5). However, there was an insignificant change in TBILI levels in mice in the control group compared with the mice administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 5). The increase in the TBILI levels was in a dose-dependent manner.

It was also observed that there was a significant decrease in DBILI levels in mice in the control group compared with mice administered with the extract at a dose of 2000 mg/kg.bw ($p<0.05$; Table 5). In addition, there was insignificant change in DBILI levels in mice in the control group compared with the mice administered with the extract ay doses of 100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 5). There was also no significant change in DBILI in mice administered with the extract at doses of 500, 1000 and 2000 mg/kg.bw ($p>0.05$; Table 5). In addition, the DBILI levels were increased in a dose-dependent manner.

Effect of DCM-MeOH extract of *Allium sativum* on kidney functions in normal mice

There was a significant decrease in BUN levels in mice in the control group compared with the mice administered with the extract a dose of 2000 mg/kg.bw ($p<0.05$; Table 6). However, there was an insignificant change in BUN levels in mice in the control group compared with the mice administered with the extract at doses of 100, 500, and 1000 mg/kg.bw; the increase in BUN levels was dose-dependent ($p>0.05$; Table 6).

There was significant increase in creatinine levels in mice administered with the extract at a dose of 100 mg/kg.bw compared with the mice in the control group ($p<0.05$; Table 6). In addition, the change in creatinine levels was insignificant among mice in the control group and mice administered with the extract at doses of 500, 1000 and 2000 mg/kg.bw ($p>0.05$; Table 6). There was also an insignificant change in creatinine among mice in the experimental groups administered with the extract at doses of 100, 500 and 1000 mg/kg.bw ($p>0.05$; Table 6). Moreover, the Cr levels increase was in a dose-dependent manner.

For Cl^- ions there was a significant decrease in mice in the control group compared with the mice administered with the extract at doses of 1000 and 2000 mg/kg.bw ($p<0.05$; Table 6). However, the change in Cl^- ions among mice in the control group and mice administered with the extract at doses of 100 and 500 mg/kg.bw was not significantly different ($p>0.05$; Table 6). In addition, there was an insignificant change in Cl^- ions in mice administered with the extract at doses of 1000 and 2000 mg/kg.bw ($p>0.05$; Table 6). Similarly, there was no significant change in Cl^- ions in mice administered with the extract at a dose of 100 mg/kg.bw compared with mice administered with the extract at a dose of 1000 mg/kg.bw ($p>0.05$; Table 6). However, the Cl^- ions increase was not in a dose-dependent manner.

Treatment	Kidney functions parameters				
(mg/kg.bw)	BUN	Cr	Cl^-	K^+	Na^+
Control	3.13 ± 0.48^b	0.50 ± 0.12^a	96.80 ± 2.37^c	3.38 ± 0.05^c	210.04 ± 4.84^b
100	3.75 ± 0.39^{ab}	0.53 ± 0.05^b	99.46 ± 1.77^{bc}	12.06 ± 1.25^a	234.28 ± 5.01^a
500	3.85 ± 0.13^{ab}	0.68 ± 0.03^{ab}	98.78 ± 1.03^c	8.77 ± 0.27^{ab}	227.78 ± 2.54^a
1000	4.32 ± 0.16^{ab}	0.72 ± 0.05^{ab}	106.78 ± 1.80^{ab}	7.47 ± 0.23^b	222.74 ± 3.95^{ab}
2000	5.06 ± 0.56^a	0.86 ± 0.01^a	108.86 ± 2.00^a	9.80 ± 1.30^{ab}	220.20 ± 4.51^{ab}

Results are expressed as Mean ± SD for five animals per group. Values with the same superscript across treatments are not significantly different from each other at $p \leq 0.05$. (Analysed by ANOVA followed by Tukey's post hoc test). Key: BUN: blood Urea Nitrogen; Cr: creatinine; Cl^-: chloride ions; K^+: potassium ions; Na^+: sodium ions.

Table 6: Effect of DCM-MeOH extract of *Allium sativum* on kidney functions in normal mice.

There was a significant increase in K^+ ions in mice in all the experimental groups compared with mice in the control group ($p<0.05$; Table 6). However, there was an insignificant change in K^+ ions among mice administered with the extract at doses of 100, 500 and 2000 mg/kg.bw ($p>0.05$; Table 6). There was also no significant change in K^+ ions in mice administered with the extract at doses of 500, 1000 and 2000 mg/kg.bw ($p>0.05$; Table 6). In addition, the K^+ ions increase was not in a dose-dependent manner.

On the other hand, there was a significant decrease in Na^+ ions in mice in the control group compared with the mice administered with the extract at doses of 100 and 500 mg/kg.bw ($P<0.05$; Table 6). However, there was no significant change in Na^+ ions in mice in the control group compared with the mice administered with the extract at doses of 1000 and 2000 mg/kg.bw. Similarly, there was an insignificant change in Na^+ ions in mice in the experimental groups administered with the extract at doses of 100, 500, 1000 and 2000 mg/kg.bw; however increase in Na^+ was not in a dose-dependent manner ($p>0.05$; Table 6).

Phytochemical Screening

Phytochemical	Presence/Absence
Alkaloids	+
Flavonoids	+
Steroids	+
Saponins	+
Cardiac glycosides	+
Phenolics	+
Terpenoids	-

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign while + (trace) denote slightly present phytochemicals

Table 7: Qualitative phytochemical screening of DCM-MeOH extract of *Allium sativum.*

Qualitative phytochemical screening of the DCM-MeOH extract of *A. sativum* revealed the presence of alkaloids, flavonoids, phenolics, steroids, saponins and cardiac glycosides. However, terpenoids were not detected in the extract (Table 7).

Discussion

The first aspect of *A. sativum* safety was how the body weight of the albino mice were affected by various administered dosages. In this study, there was a reported decrease in the body weight of the test albino mice after being administered with *A. sativum*. This was in agreement with Banerjee and Maulik [13], who observed that prolonged feeding of rats with garlic 40 and ginger 40 mg/kg may cause weight loss, anaemia and stunted growth in rat models. However, this was in contrast with the findings of Al-sarraf [14] who observed an increase in body weight of male chicks injected subcutaneously with ethanol extracted garlic at two doses of (100 and 200 mg/kg.bw).

The observed weight loss may have been as a result of minimal food and water intake, a phenomenon that is due to the feeling of fullness and appetite loss after the extract administration [15]. It may also be attributed to the triglyceride-lowering activity of *A. sativum* [16]. The anti-lipidemic effect of the extract can also be the cause the weight loss of the albino mice [17]. At 2000 mg/kg.bw dose of the extract, the average weight change per week was the highest in terms of weight loss as compared to other groups. This was likely due to increased toxicity which probably led to decreased food and water intake as reported by Joseph et al. [15] who was working with aqueous extract of *A. sativum* bulbs on mice and rats.

Deleterious effect of foreign compounds, toxins, chemicals and plant extract on blood constituents of humans and animals can easily be detected through assessment of haematological parameters. In humans and most animals, these blood indices are usually used in the diagnosis of anemic episodes. There was observed decreases in red cell indices (MCV, MCH and MCHC) in this study [18]. The observed decreases were in agreement with the findings of Corzo-Martinez et al. that indicated a significant decrease of MCH and MCV levels in fish fed with highest *A. sativum* doses of (0.45 and 0.6 g/kg).

The counts of RBC, HB and HCT increased significantly as the concentration of administered *A. sativum* extract increased. This is in agreement with Iranloye [19], who reported increases in RBC and HB concentrations in rats following 30-days of garlic consumption at a dosage of 200 mg/kg. Increase in the RBC count is associated with the blood promoting action of the garlic extract as established by Griffiths

et al. [20]; who observed that garlic is a well-known folk cure, which is rich in flavonoids such as quercetin and sulfur compounds like allyl propyl disulphide which are beneficial to humans.

Increase in RBC, HB and HCT counts may also be attributed to the stimulatory effect of garlic on particular pathways especially involving hematopoietic growth factors (cytokines). These growth factors are involved with interaction of specific receptors on the surface of hematopoietic cells thereby, regulating the proliferation and differentiation of progenitor cells and functioning of mature cells. The end product of garlic metabolism may also step up Hb synthesis and RBC production through their indirect effect on erythropoietin.

Although erythropoiesis is restricted to the bone marrow, Akgul et al. [21] reported that garlic can enhance erythropoiesis in the spleen which is termed as garlic-induced extramedullary haemopoiesis. In this study, it is assumed that increase RBC, HB and HCT counts was a result of stimulation of splenic erythropoiesis in mice by garlic components that was mediated by hypoxia which led to production of erythropoietin [22].

Moreover, it is assumed that an increase or decrease in the blood indices is as a result of a defense reaction against the administered garlic extract which takes place via erythropoiesis stimulation. This, therefore, leads to the stimulation of the kidney causing direct synthesis and secretion of erythropoietin [18].

The extract of *A. sativum* significantly increased the levels of WBC, neutrophils, lymphocytes, monocytes and PLT counts. This is in agreement with the findings of Iranloye [19], who, after 30 days of garlic administration a dosage of 200 mg/kg in rats, reported increases in total leucocyte count, neutrophils, lymphocytes, monocytes, RBC and HB concentration. A significant increase in the level of leukocytic count was also reported by Micheal et al. [23]. However, this study does not agree with that of Ugwu and Omale [24], who reported a significant decrease in the level of total WBC. The work of Swirski et al. also reported a non-significant decrease in the WBC.

Moreover, the findings of Tatfeng and Enitan [25] which reported significant increases in the level of total leucocyte count, absolute lymphocytes count, neutrophils and monocytes, eosinophils and basophils summation during their work on effects of onion and garlic (750 mg/kg/d) extracts on immunologic cells strongly confirms the findings of this study. This is in line with the findings reported by Sumiyoshi and Bjarnsholt et al. [26,27] that *A. sativum* is a stimulator of the immune functions in rats.

Garlic's role in leucopoiesis may be attributed to its ability to stimulate the production of a number of colony stimulatory factors (CSF) such as the Granulocyte-CSF, monocyte-CSF and Granulocyte-Macrophage-CSF.

Cytokines activate intracellular signaling pathways which can result to myeloid and lymphoid cells proliferation and differentiation into white blood cells [28].

Increase in the levels of WBC count may be attributed to the various phytochemical substances that possess antibiotic effects which cause the proliferation of circulating white blood cells that protect the body against teratogens as reported by Augusti [29] after confirming that there was a significant increase in total white blood count in garlic-treated animal model. Monocytes can directly be activated by microbial products and this leads to production of pro-inflammatory and with some delay of anti-inflammatory cytokines. A variety of cytokines produced by monocytes include the tumor necrosis factor (TNF), interleukin-1 and interleukin-2 [30]. On the other hand, the decreases in the level of eosinophils may be indicative of the antioxidant property of the garlic extract [31].

Increase in PLT count may be associated with the effects of garlic extract on thrombopoietin production. The chemical components in garlic or the end products of its metabolism are believed to act on thrombopoietin which is responsible for the production, proliferation and maturation of megakaryocytes and differentiation of megakaryocytes into large numbers of platelets (thrombopoiesis) [32].

In any liver cell damage, the serum or plasma levels of the ALT and AST rise. The degree of liver damage is usually determined by a rise in plasma levels of these enzymes. Hepatotoxicity leads to necrosis of the hepatocytes, increased permeability and later lead to leakage of cellular enzymes from liver cytosol into the bloodstream. In the current study, there was a significant increase in both ALT and AST enzymes upon administration of *A. sativum* at 2000 mg/kg.bw. This, therefore, indicates that the high concentration of the garlic extract may have had cytotoxic effect on the liver (hepatotoxicity) [33]. This did not agree with findings of Augusti et al. [34] who reported that lipid parameters and enzyme activities including AST, ALT and ALP in rat serum decreased significantly when fed with a diet containing 5% garlic.

The ultimate breakdown product of hemoglobin is bilirubin and is a very useful tool in the diagnosis of liver and blood disorders [35]. In this study, it was found that there was significant increase in the concentration of total and direct bilirubin. Therefore, this is indicative of hepatotoxicty, which may have been as a result of high concentration of the garlic extract. This observation is in agreement with the finding of Zbinden [36].

Creatinine is the byproduct of muscle metabolism and is derived from creatine and phosphocreatine. Serum creatinine is mostly used in indirect measure of glomerular filtration rate and is filtered and excreted by the kidney. The creatinine levels were also seen to be increasing as the concentration of the garlic extract increased. The findings of Ballet [37] argue that an increase in the creatinine levels is usually due to nephrotoxicity. This shows that the high concentrations of the garlic extract caused kidney malfunctions and, therefore, not safe. Blood urea nitrogen (BUN) is a waste product formed in the liver after protein breakdown, which is later filtered and excreted by the kidneys. The elevated levels of BUN may have been as a result of nephrotoxicity [37].

Serum electrolytes were also analysed and there was a reduced level of serum sodium as the concentration of the garlic extract increased. This maybe as a result of change in the glomerular filtration or a change in the renal blood flow [38]. Moreover, this may be caused by an interference with aldosterone secretion and/or aldosterone action on the distal tubules [39]. Ultimately, the garlic extract may have been involved in the interference of the adrenergic sodium handling thereby causing its decrease. The serum potassium levels in the experimental groups showed a significant increase as compared to the control group. This may be attributed to the alteration in the potassium transport system that may have been caused by the administration of the garlic extract [38].

In addition, after the photochemical screening, various phytochemicals were observed which included alkaloids, flavonoids, steroids, saponins, cardiac glycosides and phenolics whose biological and physiological roles have been documented by Fenwick and Hanley [40]. These phytochemicals are believed to play a major role in gene

expression, enzyme activity, stimulation of the immune system and organ related to blood cell formation (in the bone marrow) [41].

It may also be assumed that the increase in WBC counts, neutrophils, monocytes and lymphocytes was a result of the phytochemicals stimulating the immune system. Flavonoids, cardiac glycosides and alkaloids may have been responsible for the increase in the RBC count due to their antioxidant properties. This probably could have been achieved by their good free radical scavenging properties that protect the RBCs against oxidative damage. In additions, saponins which have anti-nutritive effects may have resulted in RBC turnover in this study [42].

However, an increase in the concentration of the extract, which may be assumed to cause an increase in the level of saponins, may have resulted to a decrease in red blood cell indices (MCV, MHC and MCHC). This may have exhausted the erythropoietic capacity of the mice models therefore, leading to anaemia [42].

Conclusion

In conclusion, the haematological parameters including the RBC, HB, HCT, WBC, NE, LY, PLT, PCT, MPV and PDW were observed to increase as the dose of the extract increased. Therefore, the extract may have promoted erythropoiesis, leucopoiesis and thrombopoiesis. Liver functions parameters including ALT, AST, TBILI and DBILI were observed to increase as the dosage of the extract increased. In addition, kidney parameters such as BUN and Cr were observed to increase as the dosage of the extract was increased. Electrolytes namely Cl^- and K^+ ions also increased as the dosage of the extract was increased. However, Na^+ ions were observed to decrease as the concentration increased. Thus, the extract may have hepatotoxic, nephrotoxic properties. The DCM-MeOH extract of *A. sativum* showed presence of various phytochemicals including alkaloids, flavonoids, steroids, saponins, cardiac glycosides and phenolics.

Recommendations

The extract of *A. sativum* can be safely used as an alternative medicine at dosages below 2000 mg/kg body weight. A bioassay of phytochemical fractions can be researched and developed into new drug molecules.

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